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(54) Title: TREATMENT OF CHRONIC INFLAMMATORY CONDITIONS

(57) Abstract

The invention relates to the use of antigenic and/or immunoregulatory material derived from *Mycobacterium vaccae* for use in the manufacture of a therapeutic agent for the treatment of pathological condition (other than tuberculosis, leprosy or rheumatoid arthritis) in a patient in which the patient's IgG shows an abnormally high proportion of agalactosyl IgG or in the treatment of a chronic inflammatory disorder (other than rheumatoid arthritis) caused or accompanied by an abnormally high release by macrophages of interleukin-6 and/or tumour necrosis factor.

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TREATMENT OF CHRONIC INFLAMMATORY CONDITIONS

This invention relates to the treatment of chronic inflammatory conditions, e.g. psoriasis.

British Specification No. 2156673 describes immunotherapeutic agents comprising killed cells of Mycobacterium vaccae. These agents are useful in the immunotherapy of mycobacterial disease, especially tuberculosis and leprosy. It is stated that use of this immunotherapeutic agent facilitates the removal of the persisting bacilli responsible for tuberculosis or leprosy which, as is well known, it is difficult to remove by chemotherapy alone. It is suggested in the specification that the immunotherapeutic agent is believed to act by presenting the "protective" common mycobacterial antigens to advantage and by containing immune suppressor determinants which are active in regulating disadvantageous immune mechanisms. As a consequence, "persister" bacilli are recognized by the immune system by their content of common mycobacterial antigens and effective immune mechanisms are directed against them, in the absence of the tissue necrotic form of immunity usually present in mycobacterial disease.

International Patent Specification PCT/GB 85/00183 describes compositions for the alleviation of the symptoms of, and for the treatment or diagnosis of, arthritic diseases which comprise as active ingredient the whole organism of M. vaccae. It is stated that the preparations

of M. vaccae are useful for the treatment of various autoimmune diseases and especially arthritic conditions including rheumatoid arthritis, ankylosing spondylitis or Reiter's syndrome.

We have now discovered that compositions comprising antigenic and immunoregulatory material derived from Mycobacterium vaccae are generally useful in the treatment of pathological conditions in which the proportion of agalactosyl IgG (i.e. IgG which lacks terminal galactose from the N-linked oligosaccharides on the heavy chains) is increased. Diseases of this kind include not only the rheumatoid arthritis, tuberculosis and leprosy mentioned in the specifications referred to above, but also Crohn's disease and reactive arthritis. Other diseases in which this may play a part but in which an increased level of agalactosyl IgG is not easily detectable by current methods include primary biliary cirrhosis, sarcoidosis, ulcerative colitis, psoriasis, systemic lupus erythematosus (especially when accompanied by Sjogren's syndrome), multiple sclerosis, Guillain-Barré syndrome, primary diabetes mellitus, and perhaps some aspects of graft rejection.

Such diseases may also be described as that class of chronic inflammatory disorder which is caused by, or accompanied by, abnormally high cytokine release by macrophages of interleukin-6 and/or tumour necrosis factor

(cachectin). The specific conditions involved are, of course, the same as those already named.

The present invention accordingly provides a method for the treatment of a pathological condition (other than the tuberculosis, leprosy and rheumatoid arthritis mentioned in the specifications referred to above) in a patient in which the patient's IgG shows an abnormally high proportion of agalactosyl IgG which comprises administering to the patient suffering from such a condition an effective amount of a therapeutic composition comprising antigenic and immunoregulatory material derived from Mycobacterium vaccae.

The invention also provides a method for the treatment of a chronic inflammatory disorder (other than rheumatoid arthritis) caused or accompanied by an abnormally high release from macrophages of interleukin-6 and/or tumour necrosis factor which comprises administering to a patient suffering from such a disorder an effective amount of the said therapeutic agent.

The invention further provides antigenic and immunoregulatory material derived from M. vaccae for use in the manufacture of a therapeutic agent for the treatment of pathological conditions (other than tuberculosis, leprosy or rheumatoid arthritis) in a patient whose IgG shows an abnormally high proportion of agalactosyl IgG. Such antigenic an immunoregulatory material is also provided for use in the manufacture of a therapeutic agent for use in the treatment of a chronic inflammatory disorder (other than

rheumatoid arthritis) of the kind mentioned above.

The therapeutic agent of the invention conveniently, and therefore preferably, comprises dead cells of M. vaccae, most preferably cells which have been killed by autoclaving or by irradiation. The therapeutic agent normally comprises more than 10^8 microorganisms per ml of diluent, and preferably from 10^8 to 10^{11} killed M. vaccae microorganisms per ml of diluent.

The diluent may be pyrogen-free saline for injection alone, or a borate buffer of pH 8.0. The diluent should be sterile. A suitable borate buffer is:

$Na_2B_4O_7.10H_2O$	3.63 g
H_3BO_3	5.25 g
NaCl	6.19 g
Tween 80	0.0005%
Distilled Water	to 1 litre

The preferred strain of M. vaccae is one denoted R877R isolated from mud samples from the Lango district of Central Uganda (J.L. Stanford and R.C. Paul, Ann. Soc. Belge Med, Trop. 1973, 53 141-389). The strain is a stable rough variant and belongs to the aurum sub-species. It can be identified as belonging to M. vaccae by biochemical and antigenic criteria (R. Bonicke, S.E. Juhasz., Zentr albl. Bakteriol. Parasitenkd. Infection skr. Hyg. Abt. 1, Orig., 1964, 192, 133).

The strain denoted R877R has been deposited under the Budapest Convention at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

For the preparation of the therapeutic agent, the microorganism M. vaccae may be grown on a suitable solid medium. A modified Sauton's liquid medium is preferred (S.V. Boyden and E. Sorkin., J. Immunol, 1955 75, 15) solidified with agar. Preferably the solid medium contains 1.3% agar. The medium inoculated with the microorganisms is incubated aerobically to enable growth of the microorganisms to take place, generally at 32°C for 10 days. The organisms are harvested, then weighed and suspended in a diluent. The diluent may be unbuffered saline but is preferably borate-buffered and contains a surfactant such as Tween 80 as described above. The suspension is diluted to give 100 mg of microorganism/ml. For further dilution, borate buffered saline is preferably used so that the suspension contains 10 mg wet weight of microorganisms/ml of diluent. The suspension may then be dispensed into 5 ml multidose vials. Although the microorganisms in the vials may be killed using irradiation e.g. from ^{60}Cobalt at a dose of 2.5 megarads, or by any other means, for example chemically, it is preferred to kill the microorganisms by autoclaving, for example at 10 psi (69 kPa) for 10 minutes (115°-125°C). It has been discovered, unexpectedly, that autoclaving yields a more

effective preparation than irradiation.

The therapeutic agent is in general administered by injection in a volume in the range 0.1-0.2 ml, preferably 0.1 ml, given intradermally. A single dosage will generally contain from 10^7 to 10^{10} killed M. vaccae microorganisms. It is preferred to administer to patients a single dose containing 10^8 to 10^9 killed M. vaccae. However, the dose may be repeated depending on the condition of the patient.

While the present invention does not depend on the truth of this theory it is believed that the active ingredient in the killed M. vaccae may be the 65 kDa mycobacterial heat shock protein (hsp 65) described by Young et al. "Stress proteins are immune targets in leprosy and tuberculosis", Proc. Natl. Acad. Sci. U.S.A. 85 (1988), pp4267-4270 in a form obtained from M. bovis. The preferred autoclaved M. vaccae cells used in the present invention are believed to provide an effective package of the hsp 65 and other substances in a convenient adjuvant.

Although the therapeutic agent will generally be administered by intradermal injection, other routes, e.g. oral administration, can also be used.

It may be advantageous and is within the scope of the invention to use more than one strain of M. vaccae, and/or to include in the immunoprophylactic agent other mycobacterial antigens. Tuberculin may also be included.

The immunoprophylactic agent may also contain BCG (Bacillus Calmette-Guerin) vaccine, in particular the

freeze-fried form of the vaccine, to promote its effect.

The therapeutic agent can contain further ingredients such as adjuvants, preservatives, stabilisers etc. It may be supplied in sterile injectable liquid form or in sterile freeze-fried form which is reconstituted prior to use.

M. vaccae may be used as such or as an extract or fractioned portion of the organism to manufacture the therapeutic agents according to the invention.

The following Example illustrates the invention.

EXAMPLE

M. vaccae NCTC 11659 is grown on a solid medium comprising modified Sauton's medium solidified with 1.3% agar. The medium is inoculated with the microorganism and incubated for 10 days at 32°C to enable growth of the microorganism to take place. The microorganisms are then harvested by gently scraping the surface of the agar and weighed (without drying) and suspended in M/15 borate buffered saline at pH8 to give 10 mg of microorganisms/ml of saline. The suspension is dispensed into 5 ml vials, and then autoclaved for 10 minutes at 10 psi (69 kPa) to kill the microorganisms. After cooling, 1/10th volume of tuberculin (at the standard concentration of 2 μg/ml) is added. The therapeutic agent thus produced is stored at 44°C before use. A single dose consists of 0.1 ml of the suspension, which should be shaken vigorously immediately

before use, containing 1 mg wet weight of M. vaccae and 0.02 µg of tuberculin. The dose is given by intradermal injection normally over the left deltoid muscle.

Only one dose is normally required. The patient should not receive high dose steroids or other immuno-suppressive therapy. Up to six months may elapse before the beneficial effect becomes apparent.

CLAIMS

1. Use of antigenic and/or immunoregulatory material derived from Mycobacterium vaccae for use in the manufacture of a therapeutic agent for the treatment of pathological conditions (other than tuberculosis, leprosy or rheumatoid arthritis) in a patient in which the patient's IgG shows an abnormally high proportion of agalactosyl IgG or in the treatment of a chronic inflammatory disorder (other than rheumatoid arthritis) caused or accompanied by an abnormally high release by macrophages of interleukin-6 and/or tumour necrosis factor.

2. The use according to claim 1, wherein the antigenic and/or immunoregulatory material derived from M. vaccae comprises dead cells of M. vaccae.

3. The use according to claim 2, wherein the cells of M. vaccae have been killed by autoclaving.

4. The use according to claim 1, wherein the antigenic and/or immunoregulatory material derived for M. vaccae comprises the 65 kDa heat shock protein.

5. The use according to any one of the preceding claims, wherein the material derived from M. vaccae is derived from the strain as deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659.

6. The use according to any one of the preceding claims, wherein the therapeutic agent contains,

per dose, antigenic and/or immunoregulatory material from 10^7 to 10^{10} M. vaccae microorganisms.

7. A method for the treatment of a pathological condition (other than tuberculosis, leprosy and theumatoid arthritis) in a patient in which the patient's IgG shows an abnormally high proportion of agalactosyl IgG or for the treatment of a chronic inflammatory disorder (other than rheumatoid arthritis) caused or accompanied by an abnormally high release from macrophages of interleukin-6 and/or tumour necrosis factor, which comprises administering to the patient suffering from such a condition an effective amount immunoregulatory material derived from Mycobacterium vaccae.

8. A method according to claim 7, wherein the material derived from M. vaccae is as defined in any one of claims 2 to 6.

9. Products comprising antigenic and/or immunoregulatory material derived from Mycobacterium vaccae for use in treatment of a pathological condition (other than tuberculosis, leprosy and theumatoid arthritis) in a patient in which the patient's IgG shows an abnormally high proportion of agalactosyl IgG or for the treatment of a chronic inflammatory disorder (other than rheumatoid arthritis) caused or accompanied by an abnormally high release from macrophages of interleukin-6 and/or tumour necrosis factor.

10. Products according to claim 9, wherein the material derived from M. vaccae is as defined in any one of claims 2 to 6.

11. A pharmaceutical agent for use in the treatment of a pathological condition (other than tuberculosis, leprosy and rheumatoid arthritis) in a patient in which the patient's IgG shows an abnormally high proportion of agalactosyl IgG or for the treatment of a chronic inflammatory disorder (other than rheumatoid arthritis) caused or accompanied by an abnormally high release from macrophages of interleukin-6 and/or tumour necrosis factor, which agent comprises antigenic and/or immunoregulatory material derived from Mycobacterium vaccae.

12. An agent according to claim 11, wherein the material derived from M. vaccae is as defined in any one of claims 2 to 6.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01318

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 39/04

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K, C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	WO, A, 85/05034 (UNIVERSITY COLLEGE LONDON) 21 November 1985 see the whole document	1-6,9-12
A	WO, A, 85/03639 (UNIVERSITY COLLEGE LONDON) 29 August 1985 see the whole document	1-6,9-12
A	EP, A, 0262710 (DE STAAT DER NEDERLANDEN) 6 April 1988 see the whole document	1-6,9-12
A	EP, A, 0322990 (DE STAAT DER NEDERLANDEN) 5 July 1989 see the whole document	1-6,9-12

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* Special categories of cited documents: [14]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th November 1990	18. 12. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	miss T. MORTENSEN

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	EP, A, 0045237 (BERRI BALZAC) 3 February 1982 see the whole document	1-6,9-12

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 7-8, because they relate to subject matter not required to be searched by this Authority, namely:
see rule PCT 39.1 (iv):
Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers _____, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9001318 SA 39696

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 11/12/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A- 8505034	21-11-85	AU-B-	588809	28-09-89
		AU-A-	4297685	28-11-85
		EP-A,B	0181364	21-05-86
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EP-A- 0045237	03-02-82	FR-A,B	2487198	29-01-82
		AT-T-	E8146	15-07-84
		JP-A-	57140792	31-08-82
		US-A-	4404194	13-09-83